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Clinical Importance of Superior Sensitivity of the Aptima TMA-Based Assays for *Mycoplasma genitalium* Detection

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ABSTRACT *Mycoplasma genitalium* (MG) is a common cause of nongonococcal cervicitis and urethritis. We investigated the demographic and clinical characteristics of patients tested in Denmark with the Conformité Européenne (CE)/*in vitro* diagnostics (IVD) Aptima *Mycoplasma genitalium* assay (CE/IVD AMG; Hologic) and examined the clinical significance of the higher sensitivity of the TMA-based MG assays. From March to June 2016, urogenital and extragenital specimens from consecutive attendees at a sexually transmitted infection clinic in Copenhagen, Denmark were tested with the CE/IVD AMG assay (TMA-based), the research-use-only MG Alt TMA-1 assay (Hologic), a laboratory-developed TaqMan *mgp*B quantitative real-time PCR (qPCR), and the Aptima Combo 2 (CT/NG; Hologic). Demographic characteristics and clinical symptoms were collected from the patient records. There were 1,245 patients included in the study. The MG prevalence among female subjects was 9.4%, and the MG prevalence among male subjects was 8.7%. Compared to the TMA-based assays, the sensitivity of the PCR-based MG assay was 64.52%, and 55 specimens from 48 individuals were missed in the *mgp*B qPCR. Of these, 26 individuals (54.2%) were symptomatic, whereas, among 64 individuals with concordant results, 30 individuals (46.9%) were symptomatic; no statistically significant difference was found between the groups ($P = 0.567$). The improved sensitivity of the TMA-based assays resulted in diagnoses of more patients with clinically relevant symptoms for which antibiotic treatment is indicated. However, approximately half of the MG-infected patients reported no symptoms, and future research is needed to investigate the pros and cons of diagnosing and treating MG in asymptomatic subjects.

KEYWORDS Aptima, *Mycoplasma genitalium*

Mycoplasma genitalium (MG) is a common cause of nongonococcal urethritis (NGU) in men and is associated with cervicitis, pelvic inflammatory disease, endometritis, and probably infertility in women (1–6). In addition to the urogenital sites, MG can also be found in the rectum and, very rarely, in the oropharynx and eye (7–11). The Nordic Aptima MG Evaluation (NAME) study from 2016 found a high prevalence of MG in Denmark, Norway, and Sweden: 9.0%, 4.9%, and 9.8%, respectively (12). In all countries, the prevalence was slightly higher among women (12). The NAME study was designed to evaluate the clinical and analytical performance of the Conformité Européenne (CE)/*in vitro* diagnostics (IVD) Aptima *Mycoplasma genitalium* assay (CE/IVD AMG; Hologic), which now is also U.S. FDA-approved, compared to the research-use-only MG Alt TMA-1 assay (Hologic) and a laboratory-developed TaqMan *mgp*B quantitative real-time PCR (qPCR). The study included both symptomatic and asymptomatic individuals and found that the two transcription-mediated amplification (TMA)-based MG assays from Hologic had a highly

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superior sensitivity compared to the *mgp*B qPCR, i.e., the sensitivity was 99.13−100%, 99.13−100%, and 73.24−81.60%, respectively (12).

Increasing rates of resistance in MG, both to first-line (azithromycin) and second-line (moxifloxacin) treatment, has also been of concern (1, 4, 5, 12). The NAME study found high rates of resistance-associated mutations of 41.4% for azithromycin and 6.6% for moxifloxacin, with large differences between study sites (12). Dual resistance was also found in all countries, i.e., Denmark, Norway, and Sweden (4.2%, 1.1%, and 3.1%, respectively) (12).

The aim of the present study was to examine the demographic and clinical characteristics of the patients from the NAME study (12) included in Denmark and to investigate the clinical significance of the higher sensitivity of the TMA-based MG assays (CE/IVD AMG and MG Alt TMA-1) compared to the well-recognized laboratory-developed *mgp*B qPCR (13, 14).

MATERIALS AND METHODS

Patients. Consecutive female and male subjects attending the sexually transmitted infection (STI) clinic at Bispebjerg Hospital, Copenhagen University Hospital, Denmark, from March to June 2016 were enrolled into the study. All patients ≥18 years with a Danish Personal Identification Number (PIN) were eligible, and only patients who declined to participate were excluded. The STI clinic is the largest in Denmark and is attended by approximately 100 patients daily. Individuals attended the clinic because they had symptoms, because they had had unprotected sex, or because they had received a partner notification. All patient records were reviewed, and the following data were extracted: symptoms of an STI, sociodemographic information, sexual orientation (e.g., men who have sex with men [MSM] status), HIV status, and sexual risk behavior (e.g., sex work or having sex with a sex worker or a person with an STI).

Specimens. Specimens from male and female subjects were collected according to clinical indication and risk profile. Gynecological examinations were performed on symptomatic women to collect urethral and cervical swabs. In addition, clinicians collected vaginal swab samples for this study. Asymptomatic women self-collected two vaginal swabs simultaneously and placed them in separate tubes. All MSM were tested from urethral, rectal, and pharyngeal sites regardless of symptoms. Men who have sex with women were tested from urethra. In addition, rectal and pharyngeal swabs were collected from female and male subjects as indicated by clinical evaluation and risk profile.

Urogenital and extragenital specimens were tested with the CE/IVD AMG assay (Hologic), the research-use-only MG Alt TMA-1 assay (Hologic) (12), the *mgp*B qPCR (13, 14), and the Aptima Combo 2 assay (Hologic), which detects *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (12).

Definitions. Specimens were considered true positive if they were positive in a minimum of two of the three MG nucleic acid amplification tests (NAATs). The study subjects were categorized as MG-infected (minimum one true positive specimen) or not MG-infected (no positive specimens). To investigate the clinical importance of the superior sensitivity of the TMA-based MG assays compared to the *mgp*B qPCR, specimens positive in the TMA-based assays and negative in the *mgp*B qPCR were investigated further as a separate group and designated as specimens with discrepant results. Female subjects were categorized as symptomatic if they reported rectal itching/discharge, genital itching, abnormal discharge, or pain/discomfort in the lower abdomen or during urination. Likewise, male subjects were categorized as symptomatic if they reported rectal itching/discharge, genital itching, abnormal discharge, or pain/discomfort during urination.

Statistical analysis. Categorical data were compared using χ^2 test or Fisher's exact test, where appropriate. The Mann-Whitney test was used for comparison of the bacterial load. IBM SPSS Statistics software was used with significance set at $P < 0.05$.

Ethics. The study was approved by the Danish Data Protection Agency (journal number 2012-58-0004/BFH-2016-006) and the Committee on Health Research Ethics (journal number H-15018251). All study subjects provided written informed consent prior to specimen collection. Participants were not compensated for study participation.

RESULTS

***Mycoplasma genitalium*-positive samples.** In total, 1,245 subjects were included, of which 499 (40.1%) were female and 746 (59.9%) were male (Table 1). The median age (range) of the female subjects was 25 (18–69) years, and the median age (range) of the male subjects was 32 (18–70) years. Based on self-reported sexual orientation, 31.1% of the men were MSM. The number of MG-infected subjects was 112 (9.0%) based on the definition of presenting a minimum of two out of three positive MG NAATs in at least one specimen. The overall prevalence among female subjects was 9.4% (Table 1), and the prevalence was 9.8% and 9.0% among symptomatic and asymptomatic female subjects, respectively ($P = 0.878$). Among male subjects, the overall prevalence was 8.7% (Table 1), and the prevalence was 11.2% and 7.3% among symptomatic and asymptomatic males, respectively ($P = 0.081$). In female subjects, the

TABLE 1 Prevalence of *Mycoplasma genitalium* among 1,245 individuals

Category	Female		Male	
	No. of positive / total no.	No. of negative / total no.	No. of positive / total no.	No. of negative / total no.
No. of participants	47/499 (9.4%)	452/499 (90.6%)	65/746 (8.7%)	681/746 (91.3%)
Symptomatic	25/47 (53.2%)	230/452 (50.9%)	31/65 (47.7%)	247/681 (36.3%)
WSW or MSM[a]	3/36[b] (8.3%)	35/394[c] (8.9%)	25/62[d] (40.3%)	202/667[e] (30.3%)

[a]WSW, women who have sex with women; MSM, men who have sex with men.
[b]Information not available for 11 individuals.
[c]Information not available for 58 individuals.
[d]Information not available for 3 individuals.
[e]Information not available for 14 individuals.

prevalence was lowest (0%) in pharyngeal specimens from both asymptomatic and symptomatic individuals and highest (8.2%) in urethral specimens from symptomatic individuals. For comparison, the prevalence was 7.1% in both vaginal and cervical specimens from symptomatic female subjects, whereas there was no statistically significant difference from the urethral specimens ($P = 0.739$). In male subjects, the prevalence was lowest (0%) in pharyngeal specimens from symptomatic individuals and highest (10.4%) in urethral specimens from symptomatic individuals. Further, based on self-reported sexual orientation, 40.3% of the MG-infected male subjects were MSM, which was not significantly different from the MG-negative population where 30.3% were MSM ($P = 0.115$, Table 1).

Coinfection with *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*. Six MG-infected subjects were coinfected with *C. trachomatis* (5.4%), and seven MG-infected subjects were coinfected with *N. gonorrhoeae* (6.3%), including two individuals with all three infections concomitant. To evaluate whether symptom status was influenced by *C. trachomatis* and/or *N. gonorrhoeae* coinfection, analyses were repeated without coinfected subjects. Among these 101 subjects with MG-mono-infection, 54.8% of the female subjects were symptomatic compared to 47.4% of the negative female subjects ($P = 0.419$). Likewise, 44.1% of the MG-mono-infected male subjects were symptomatic compared to 33.8% of the negative male subjects ($P = 0.117$).

Comparison of the TMA-based MG assays and the mgpB qPCR. All specimens positive in the MG Alt TMA-1 assay were also positive in the CE/IVD AMG assay. Compared to the TMA-based assays, the sensitivity of the *mgp*B qPCR assay was 64.52% on a specimen level. Fifty-five specimens were positive in the two TMA-based MG assays and negative in the *mgp*B qPCR (Tables 2 and 3). These 55 specimens with discrepant results originated from 48 individuals, of whom 41 individuals had only one specimen with discrepant results and seven individuals had two specimens with discrepant results. However, 16 (33%) of the 48 individuals with specimens with discrepant results had specimens from other sites that were positive in all three assays. Among the 48 individuals with discrepant results, 26 had symptoms (54.2%), whereas, among the 64 individuals with concordant results, 30 had symptoms (46.9%), and there was no statistically significant difference between the groups ($P = 0.567$). Among the individuals with concordant results (and thereby positive in the *mgp*B qPCR), we found that symptomatic individuals had significantly higher bacterial loads compared to asymptomatic individuals ($P = 0.038$). Further, we stratified all MG-mono-infected individuals according to the severity of symptoms (pain/discomfort, abnormal discharge/pain during urination, and discomfort/itching) and found that minor symptoms such as itching and discomfort were more prevalent among individuals with discrepant results (21%) compared to individuals with concordant results (10%) ($P = 0.001$).

When comparing specimens with discrepant results with specimens with positive results in all three assays, significantly more specimens from MSM were missed in the *mgp*B qPCR ($P = 0.028$, Table 4), and this trend was also observed when looking only at the urethral specimens ($P = 0.058$, Table 4) but not in the pharyngeal and rectal specimens. Of note, very few heterosexual men had specimens taken from pharynx and

TABLE 2 Symptom status of female subjects

Location and symptom status	No. of specimens positive in three assays	No. of specimens with discrepant results[a]	No. of specimens negative in three assays	No. of specimens collected in total	Specimens not collected
Pharynx					
Sym	0 (0%)	0 (0%)	33 (100%)	33	222
Asym	0 (0%)	0 (0%)	38 (100%)	38	206
Total	0 (0%)	0 (0%)	71 (100%)	71	428
Urethra					
Sym	10 (4%)	11 (5%)	216 (91%)	237	18
Asym	2 (4%)	2 (4%)	47 (92%)	51	192
Total	12 (4%)	13 (5%)	263 (91%)	288	210
Rectum					
Sym	1 (5%)	1 (5%)	17 (90%)	19	236
Asym	0 (0%)	1 (6%)	16 (94%)	17	227
Total	1 (3%)	2 (5%)	33 (92%)	36	463
Vagina					
Sym	14 (7%)	4 (2%)	177 (91%)	195	59
Asym	15 (7%)	3 (1%)	208 (92%)	226	18
Total	29 (7%)	7 (2%)	385 (91%)	421	77
Cervix					
Sym	14 (6%)	4 (2%)	211 (92%)	229	24
Asym	3 (6%)	1 (2%)	44 (92%)	48	196
Total	17 (6%)	5 (2%)	255 (92%)	277	220
Total					
Sym	39 (5%)	20 (3%)	654 (92%)	713	559
Asym	20 (5%)	7 (2%)	353 (93%)	380	839
Total	59 (5%)	27 (3%)	1007 (92%)	1093	1398

[a]Defined as positive in the CE/IVD AMG and the Alt TMA-1 assays and negative in the *mgp*B qPCR.

rectum, and all these specimens were negative. When investigating the 13 male subjects with rectal MG infection, all were MSM (Table 4), three of whom were coinfected with HIV and only three having positive specimens from other sites, e.g., urethra. Three female subjects had rectal MG infection (Table 2), and all had the MG infection confirmed by positive cervical specimens. Also, when investigating the subjects with

TABLE 3 Symptom status of male subjects

Location and symptom status	No. of specimens positive in three assays	No. of specimens with discrepant results[a]	No. of specimens negative in three assays	No. of specimens collected in total	Specimens not collected
Pharynx					
Sym	0 (0%)	0 (0%)	90 (100%)	90	188
Asym	1 (1%)	3 (2%)	159 (97%)	163	304
Total	1 (1%)	3 (1%)	249 (98%)	253	492
Urethra					
Sym	21 (8%)	8 (3%)	237 (89%)	266	10
Asym	14 (3%)	11 (2%)	434 (95%)	459	6
Total	35 (5%)	19 (3%)	671 (93%)	725	16
Rectum					
Sym	0 (0%)	2 (2%)	83 (98%)	85	193
Asym	7 (5%)	4 (3%)	137 (92%)	148	319
Total	7 (3%)	6 (3%)	220 (94%)	233	512
Total					
Sym	21 (5%)	10 (2%)	410 (93%)	441	391
Asym	22 (3%)	18 (2%)	730 (95%)	770	629
Total	43 (4%)	28 (2%)	1140 (94%)	1211	1020

[a]Defined as positive in the CE/IVD AMG and the Alt TMA-1 assays and negative in the *mgp*B qPCR.

TABLE 4 MSM status of male subjects

Location and symptom status[b]	No. of specimens positive in three assays	No. of specimens with discrepant results[a]	No. of specimens negative in three assays	Specimens not collected
Pharynx				
MSM	1 (100%)	3 (100%)	218 (88%)	5 (1%)
MSW	0 (0%)	0 (0%)	31 (12%)	488 (99%)
Total	1 (100%)	3 (100%)	249 (100%)	493 (100%)
Urethra				
MSM	6 (17%)	8 (42%)	202 (30%)	8 (50%)
MSW	29 (83%)	11 (58%)	470 (70%)	8 (50%)
Total	35 (100%)	19 (100%)	672 (100%)	16 (100%)
Rectum				
MSM	7 (100%)	6 (100%)	202 (92%)	12 (2%)
MSW	0 (0%)	0 (0%)	18 (8%)	501 (98%)
Total	7 (100%)	6 (100%)	220 (100%)	513 (100%)
Total				
MSM	14 (33%)	17 (61%)	622 (55%)	25 (2%)
MSW	29 (67%)	11 (39%)	519 (45%)	997 (98%)
Total	43 (100%)	28 (100%)	1141 (100%)	1022 (100%)

[a]Defined as positive in the CE/IVD AMG and the Alt TMA-1 assays and negative in the *mgp*B qPCR.
[b]MSM, men who have sex with men; MSW, men who have sex with women.

pharyngeal MG infection, all were MSM (Table 4) and all were positive in more than one specimen type. For example, all three subjects with discrepant results in the pharyngeal specimens were positive in all three assays in the urethral and/or rectal specimens. When looking at specimens positive in the CE/IVD AMG assay and negative in the research-use-only MG Alt TMA-1 assay, 12 specimens from five male and seven female subjects were identified (two rectal, five urethral, two cervical, and three vaginal specimens). One of the men (20%) and six of the women (86%) had MG-positive specimens from other sites ($P = 0.047$). Of the five men, two were MSM, and the specimens included one rectal and one urethral specimen.

DISCUSSION

In this study we investigated the clinical characteristics of patients diagnosed with MG in a Danish STI clinic and found a high MG prevalence in both symptomatic and asymptomatic subjects. Approximately 50% of the patients defined as MG-infected reported symptoms. This number is in line with findings among other STI clinic attendees, where 40–75% of the MG-infected study subjects were asymptomatic (15, 16). However, other studies have demonstrated that up to 70% of male STI attendees diagnosed with MG were symptomatic (17). The prevalence of MG in this current study was highest in urethral specimens from symptomatic male and female subjects. Similarly, a recent multicenter study performed in the United States found the highest prevalence in urine and vaginal specimens from symptomatic female subjects and in urethral swab and urine specimens from male subjects (18). The current 2016 European guideline on *Mycoplasma genitalium* infections recommends that patients with symptoms be tested for MG (4). This strategy seems reasonable since testing of all attendees at STI clinics regardless of symptoms would cause an increase in the use of antibiotics to treat asymptomatic infections, and there is currently not enough evidence that treatment of asymptomatic infections provide benefit for the individual patient. On the other hand, treatment of MG infection in all individuals would decrease the risk of transmission and likely reduce the risk of complications, including PID (pelvic inflammatory disease) (19) and tubal factor infertility (2).

Based on results from the NAME study that demonstrated higher sensitivity of TMA-based MG assays compared to *mgp*B qPCR (12), we investigated the importance of this increased sensitivity. The specimens missed in the *mgp*B qPCR were from symptomatic subjects to the same degree as specimens from MG-infected subjects overall,

demonstrating that the increased sensitivity of the TMA-based MG assays did not result in diagnosis of solely more asymptomatic individuals. Also, most of the subjects with discrepant specimens had other specimen types that were positive in all three assays, indicating that the decreased sensitivity of the *mgp*B qPCR was not caused by infection with a certain strain, but more likely caused by lower bacterial loads in some specimens. Among the individuals with concordant results, we also found that symptomatic individuals had significantly higher bacterial loads compared to asymptomatic individuals. Similarly, the NAME study found that the *mgp*B qPCR missed the two samples with the lowest MG concentrations from an external quality assurance program, whereas it was detected by the TMA-based MG assays (12).

We found that statistically significantly more of the specimens missed in the *mgp*B qPCR were from MSM, and a similar trend was found when looking specifically at urethral specimens. Since all men, regardless of sexual practice, had specimens taken from the urethra, these results indicate that MSM may carry lower loads of *M. genitalium* or, in the worst case, are infected with other types of mycoplasmas. Indeed, the NAME study found that all strains of *M. pirum*, *M. alvi*, and *M. amphoriforme* were detected even in low concentrations in the CE/IVD AMG assay (12). However, none of these species were detected in the Alt-TMA assay and discordance between the two TMA assays was extremely rare. The knowledge about these mycoplasma species and their prevalence in human specimens is limited (12); for example, *M. pirum* has been found in rectal specimens from MSM (20) and is considered an HIV-associated mycoplasma species (21), *M. alvi* has been found mainly in the bovine gastrointestinal tract (22), and *M. amphoriforme* has been found in respiratory tract specimens from patients with primary antibody deficiency (23). In our study, all male subjects with positive rectal specimens were MSM and less than a third of these had MG infection confirmed in specimens from other sites. We hypothesize that MSM may carry lower loads of MG because of more frequent testing and antibiotic treatment of STIs, an assumption that is supported by a retrospective case-control study including individuals from the present STI clinic. The study included patients coinfected with *C. trachomatis* and *N. gonorrhoeae* and found that MSM were more often coinfected (24), supporting the recommendation of STI testing 2–4 times a year of MSM who are sexually active. Of note, pre-exposure prophylaxis (PrEP) as a general offer was introduced in Denmark in January 2019; before this, PrEP was only available to individuals enrolled in clinical trials. Information on PrEP status of the MSM in this study was incomplete, but since the specimens were collected in 2016, we assume that it was a relatively low number. Taken together, a positive CE/IVD AMG rectal test, particularly in HIV-positive MSM, should be treated with caution without confirmatory testing (12); however, in our study only one male subject had a positive CE/IVD AMG rectal test that was negative in the research-use-only MG Alt TMA-1 assay, making it difficult to draw conclusions. Moreover, the subjects with positive pharyngeal specimens all were MSM, but all subjects also had positive specimens from other sites, supporting true infection with MG. For example, all three subjects with discrepant results in the pharyngeal specimens were positive in all three assays in the urethral and/or rectal specimens, indicating that the bacterial load is lower at the pharyngeal site or that correct specimen collection is particularly difficult there. However, very few heterosexual men had specimens taken from the pharynx and rectum, which may bias these conclusions.

This study has several strengths, including our access to medical records, from all study subjects, that include symptoms, sexual orientation, and HIV status, and the linking of laboratory data by the unique PIN assigned to all Danish citizens. Also, testing was done from both urogenital and extragenital sites from consecutive symptomatic and asymptomatic study subjects. Additionally, all study subjects were tested for *C. trachomatis* and *N. gonorrhoeae* coinfection to exclude that symptom status was influenced by coinfections; accordingly, the analyses were done on both the MG-monoinfected subjects and on all the study subjects. Our study also has some limitations.

First, even though the study included more than 100 MG-infected study subjects, numbers were small when investigating specific characteristics such as specimen location and MSM status. Therefore, some associations may have been overestimated and others not detected. Second, information on sexual risk behavior, e.g., number of sexual partners and sexual risk behavior, was incomplete in many records and therefore not included in the present paper.

In summary, the improved sensitivity of the CE/IVD AMG assay resulted in the diagnosis of more MG-infected subjects with clinically relevant symptoms. As this assay is performed on the fully automated Panther system, it can easily be combined with testing for *C. trachomatis*, *N. gonorrhoeae*, and also *Trichomonas vaginalis*, allowing the simultaneous detection of MG with other genital pathogens. However, future research is needed to investigate the significance of MG in asymptomatic individuals in order to balance the benefits of diagnosis and treatment, including the reduced risk of sequelae and further transmission, against increased use of antibiotics, resistance development, potential side effects, and costs.

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REFERENCES

1. Taylor-Robinson D, Jensen JS. 2011. *Mycoplasma genitalium*: from chrysalis to multicolored butterfly. Clin Microbiol Rev 24:498–514. https://doi.org/10.1128/CMR.00006-11.
2. Lis R, Rowhani-Rahbar A, Manhart LE. 2015. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis 61:418–426. https://doi.org/10.1093/cid/civ312.
3. Horner PJ, Blee K, Falk L, van der Meijden W, Moi H. 2016. 2016 European guideline on the management of non-gonococcal urethritis. Int J STD AIDS 27:928–937. https://doi.org/10.1177/0956462416648585.
4. Jensen JS, Cusini M, Gomberg M, Moi H. 2016. 2016 European guideline on *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol 30:1650–1656. https://doi.org/10.1111/jdv.13849.
5. Unemo M, Jensen JS. 2017. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and *Mycoplasma genitalium*. Nat Rev Urol 14:139–152. https://doi.org/10.1038/nrurol.2016.268.
6. Sonnenberg P, Ison CA, Clifton S, Field N, Tanton C, Soldan K, Beddows S, Alexander S, Khanom R, Saunders P, Copas AJ, Wellings K, Mercer CH, Johnson AM. 2015. Epidemiology of *Mycoplasma genitalium* in British men and women aged 16–44 years: evidence from the third national Survey of Sexual Attitudes and Lifestyles (Natsal-3). Int J Epidemiol 44:1982–1994. https://doi.org/10.1093/ije/dyv194.
7. Francis SC, Kent CK, Klausner JD, Rauch L, Kohn R, Hardick A, Gaydos CA. 2008. Prevalence of rectal *Trichomonas vaginalis* and *Mycoplasma genitalium* in male patients at the San Francisco STD clinic, 2005–2006. Sex Transm Dis 35:797–800. https://doi.org/10.1097/OLQ.0b013e318177ec39.
8. Soni S, Alexander S, Verlander N, Saunders P, Richardson D, Fisher M, Ison C. 2010. The prevalence of urethral and rectal *Mycoplasma genitalium* and its associations in men who have sex with men attending a genitourinary medicine clinic. Sex Transm Infect 86:21–24. https://doi.org/10.1136/sti.2009.038190.
9. Nakashima K, Shigehara K, Kawaguchi S, Wakatsuki A, Kobori Y, Nakashima K, Ishii Y, Shimamura M, Sasagawa T, Kitagawa Y, Mizokami A, Namiki M. 2014. Prevalence of human papillomavirus infection in the oropharynx and urine among sexually active men: a comparative study of infection by papillomavirus and other organisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma* spp., and *Ureaplasma* spp. BMC Infect Dis 14:43. https://doi.org/10.1186/1471-2334-14-43.
10. Latimer RL, Vodstrcil L, De Petra V, Fairley CK, Read TRH, Williamson D, Doyle M, Chow EPF, Bradshaw C. 2020. Extragenital *Mycoplasma genitalium* infections among men who have sex with men. Sex Transm Infect 96:10–18. https://doi.org/10.1136/sextrans-2019-054058.
11. Mordhorst I, Potthoff A, Brockmeyer NH. 2021. Positiver *Mycoplasma genitalium*-Befund im Auge: ein Fall aus der Praxis. JDDG - J Ger Soc Dermatology 19:34–36. https://doi.org/10.1111/ddg.14482.
12. Unemo M, Salado-Rasmussen K, Hansen M, Olsen AO, Falk M, Golparian D, Aasterød M, Ringlander J, Nilsson CS, Sundqvist M, Schønning K, Moi H, Westh H, Jensen JS. 2018. Clinical and analytical evaluation of the new Aptima *Mycoplasma genitalium* assay, with data on *M. genitalium* prevalence and antimicrobial resistance in *M. genitalium* in Denmark, Norway and Sweden in 2016. Clin Microbiol Infect 24:533–539. https://doi.org/10.1016/j.cmi.2017.09.006.
13. Jensen JS. 2012. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. Methods Mol Biol 903:129–139. https://doi.org/10.1007/978-1-61779-937-2_8.
14. Jensen JS, Björnelius E, Dohn B, Lidbrink P. 2004. Use of TaqMan 5′ nuclease real-time PCR for quantitative detection of *Mycoplasma genitalium* DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol 42:683–692. https://doi.org/10.1128/JCM.42.2.683-692.2004.
15. Anagrius C, Loré B, Jensen JS. 2005. *Mycoplasma genitalium*: prevalence, clinical significance, and transmission. Sex Transm Infect 81:458–462. https://doi.org/10.1136/sti.2004.012062.
16. Falk L, Fredlund H, Jensen JS. 2005. Signs and symptoms of urethritis and cervicitis among women with or without *Mycoplasma genitalium* or *Chlamydia trachomatis* infection. Sex Transm Infect 81:73–78. https://doi.org/10.1136/sti.2004.010439.
17. Falk L, Fredlund H, Jensen JS. 2004. Symptomatic urethritis is more prevalent in men infected with *Mycoplasma genitalium* than with *Chlamydia trachomatis*. Sex Transm Infect 80:289–293. https://doi.org/10.1136/sti.2003.006817.
18. Gaydos CA, Manhart LE, Taylor SN, Lillis RA, Hook EW, Klausner JD, Remillard CV, Love M, McKinney B, Getman DK. 2019. Molecular testing for *Mycoplasma genitalium* in the United States: results from the AMES prospective multicenter clinical study. J Clin Microbiol 57:e01125-19. https://doi.org/10.1128/JCM.01125-19.
19. Oakeshott P, Aghaizu A, Hay P, Reid F, Kerry S, Atherton H, Simms I, Taylor-Robinson D, Dohn B, Jensen JS. 2010. Is *Mycoplasma genitalium* in women the "new chlamydia?" A community-based prospective cohort study. Clin Infect Dis 51:1160–1166. https://doi.org/10.1086/656739.
20. Taylor-Robinson D, Gilroy CB, Keane FE. 2003. Detection of several *Mycoplasma* species at various anatomical sites of homosexual men. Eur J

Clin Microbiol Infect Dis 22:291–293. https://doi.org/10.1007/s10096-003-0910-x.
21. Wu JR, Wang B, Chen LS, Yang T, Zhou LJ, Xie YX, Xu JS, Guo HX, Huan XP. 2014. Alarming incidence of genital mycoplasmas among HIV-1-infected MSM in Jiangsu, China. Eur J Clin Microbiol Infect Dis 33:189–195. https://doi.org/10.1007/s10096-013-1942-5.
22. Gourlay R, Wyld S, Leach R. 1977. *Mycoplasma alvi*, a new species from bovine intestinal and urogenital tracts. Int J Syst Bacteriol 27:86–96. https://doi.org/10.1099/00207713-27-2-86.
23. Pitcher DG, Windsor D, Windsor H, Bradbury JM, Yavari C, Jensen JS, Ling C, Webster D. 2005. *Mycoplasma amphoriforme* sp. nov., isolated from a patient with chronic bronchopneumonia. Int J Syst Evol Microbiol 55:2589–2594. https://doi.org/10.1099/ijs.0.63269-0.
24. Barati Sedeh F, Thomsen SF, Larsen HK, Westh H, Salado-Rasmussen K. 2021. Sex-associated risk factors for co-infection with *Chlamydia trachomatis* and *Neisseria gonorrhoea* among patients presenting to a sexually transmitted infection clinic. Acta Derm Venereol 101:adv00356. https://doi.org/10.2340/00015555-3721.